# Rational Design of Macrolides by Virtual Screening of Combinatorial Libraries Generated through in Silico Manipulation of Polyketide Synthases

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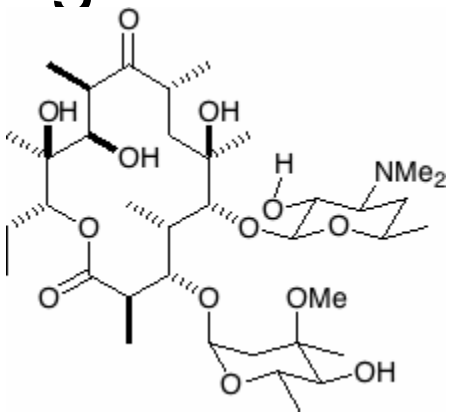
Journal of Medicinal Chemistry, 2006, 49, 2077-2087

# Outline

- Introduction
- Program Approach
- PKS Review
- Software
- Examples

# Introduction

- Natural therapeutic products
  - Random screening
- Limitations
  - Focusing on particular targets
  - Low ratio of active to inactive molecules
- Already have library building programs
  - Based on chemoinformatics
  - Target- and Ligand-based



# Program Approach

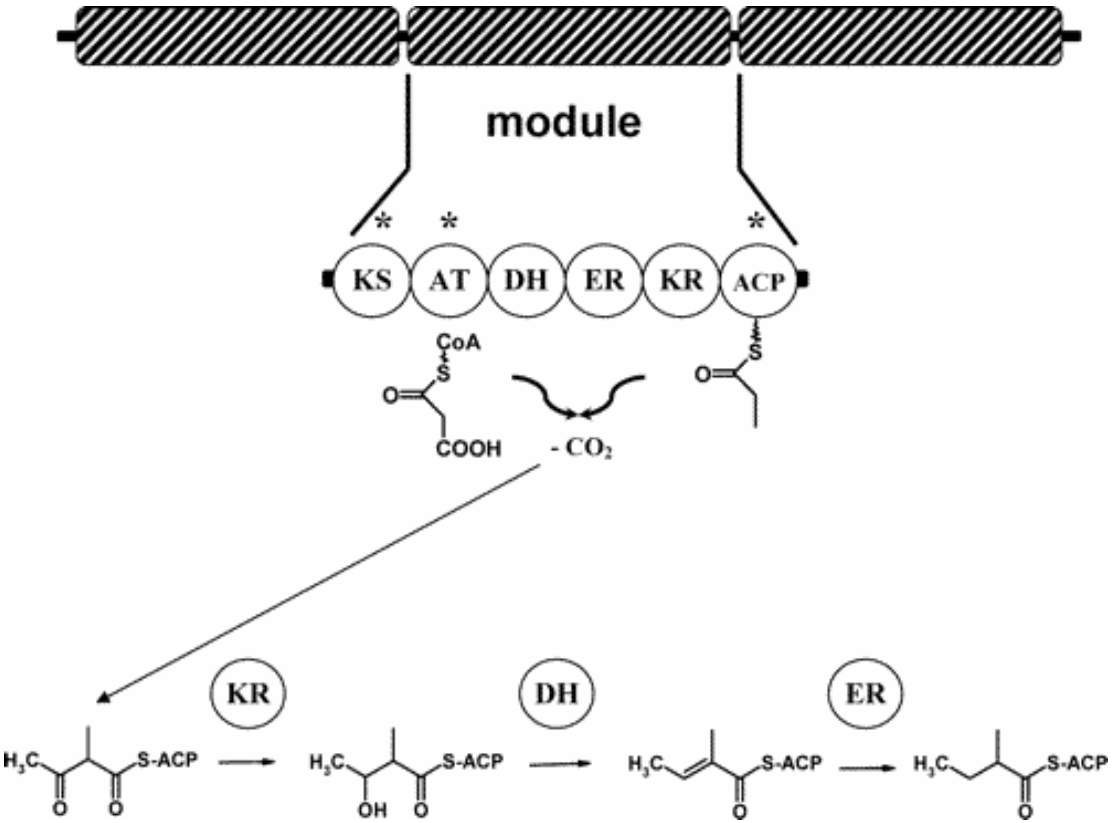
- Based on postgenomic technology and methods of chemoinformatics
- Generates secondary metabolites' structures in correspondence with the appropriate biosynthetic pathways
- Prediction of properties (biological activity, physiochemical properties, etc)

# Approach Testing

- Tested on macrolides
- All combinatorial manipulations:
  - Module and domain insertions/deletions
  - Site specific mutagenesis
- Can screen for products with desired therapeutic properties and rule out toxic products

# Review: Biosynthesis of Macrolides by Polyketide Synthases

- Polyketide synthase type I enzymes make polyketide chains
- Chains are formed from decarboxylative condensations of carboxylic acids
- Starter units are activated carboxylic acids
- Macrolactone rings formed by cyclization of polyketide chains




KS: β- ketoacylsynthase

AT: acyltransferase

KR: ketoreductase

DH: dehydratase

ER: enoyl reductase

ACP: acyl carrier protein

TE: thioesterase

Figure 1, J. Med. Chem, 2006, Vol. 49, No 6

# Successful Manipulations

- Deletions of PKS modules
- Exchanges of AT domains
- Inactivation and/or addition of reductive domains

# Problems

- Actually performing these changes in a lab can lead to
  - Loss of functionality
  - Loss of or no product yield
  - Loss of time
  - Loss of money
- Domain changes only affect the macrolactone ring which is not the final product
- Making every change is impossible

# Biogenerator Software

- Performs in silico manipulation of theoretical PKS type I systems
- Creates virtual libraries of macrolide analogs
- Introduces post-PKS modifications
- Virtual screenings for characteristics

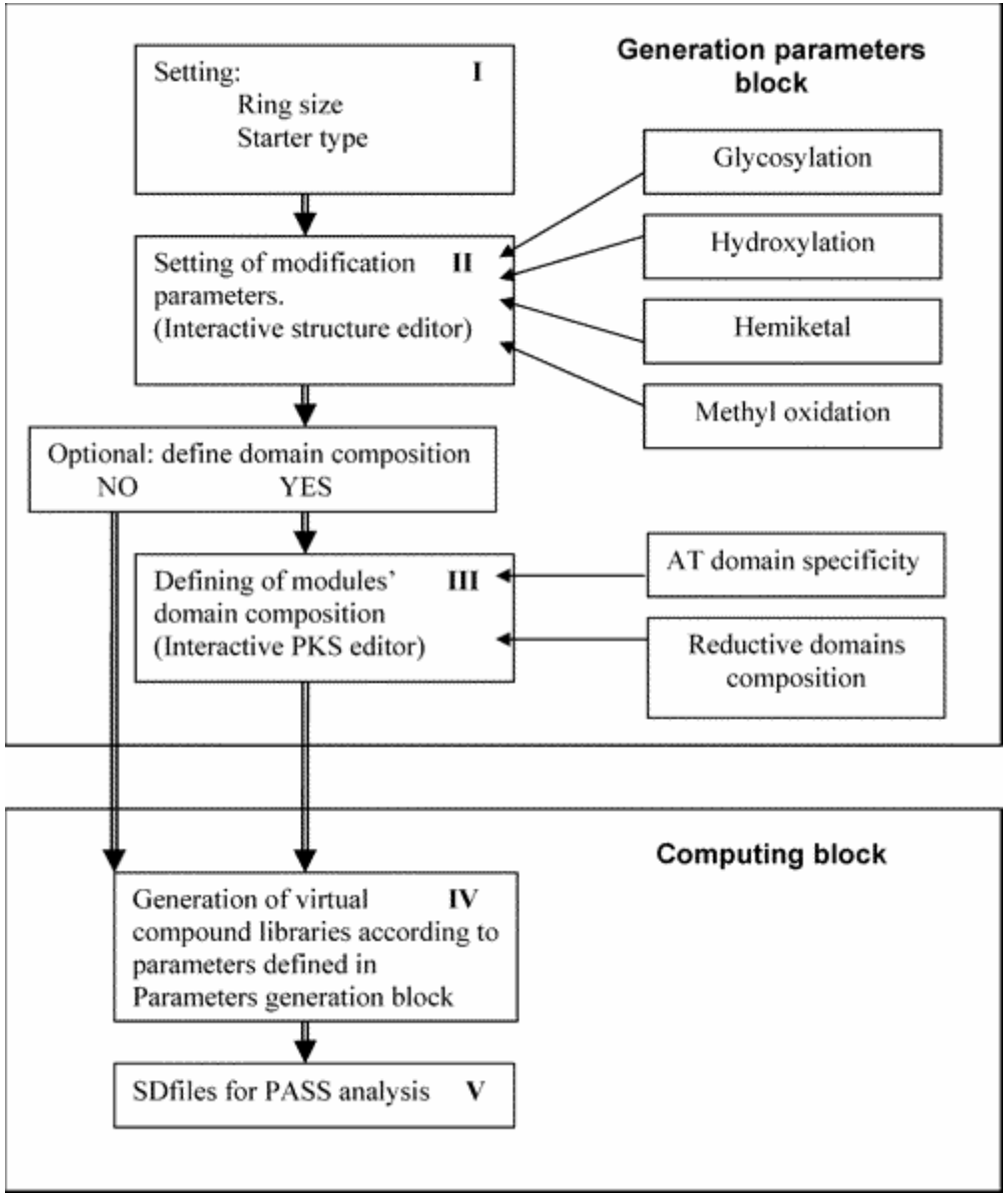



Figure 2, J. Med. Chem, 2006, Vol. 49, No 6

# Blocks

- Block I
  - Starter Type: Acetate or propionate
  - Ring Size: Up to 38 atoms (module number)
- Block II
  - Post-PKS Modification Parameters:
    - Glycosylation
    - Hydroxylation
    - Methyl Oxidation
    - Hemiketal

# Blocks

- Glycosylation: Set glycosylation sites at the putative hydroxyl groups, choose type of sugar
- Hydroxylation: Sites can be set based on knowledge of specificity of enzymes
- Methyl Oxidation: Choose if a carboxyl group is at a certain position
- Hemiketal: Restricts domain composition in two modules

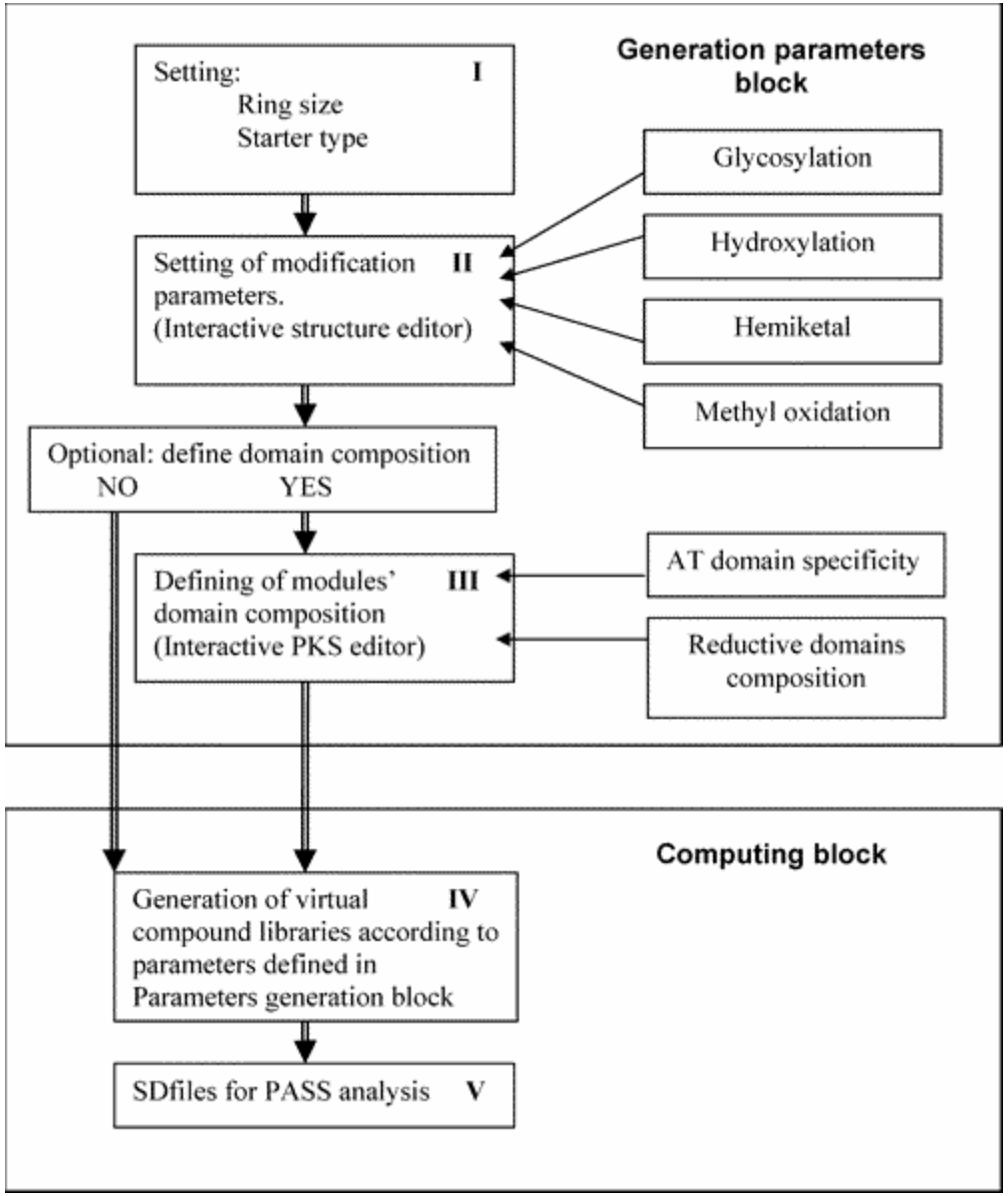



Figure 2, J. Med. Chem, 2006, Vol. 49, No 6

# Blocks

- Option to Define Domain Composition
- Block III
  - Module's Domain Composition: AT domain specificity and reductive domain composition
- Block IV
  - Generation of Virtual Combinatorial Libraries: Chemical structures and PKS notation

# Biological Activity Prediction

- PASS Software:
- 85% accuracy
- Can predict more than 2000 pharmacological effects, mechanisms of action and toxic effects based on structural formulas
- Training set of about 58,500 known substances

# Biological Activity

- Probability (Values from 0.000 to 1.000)
  - Pa: Probability to be active
  - Pi:  Probability to be inactive
- User defines threshold (T) for activities with Pa > T to be considered, default Pa=Pi
- PharmaExpert: Helps in more specific selection

# Example 1: Erythromycin

- Chosen because it has already been very well studied (control)
- Tested PASS system on 285 erythromycin analogs with experimentally confirmed activities

| Type of activity | *N* | ± | Pa | Pi |
|---|---|---|---|---|
| Acne therapy | 7 | 0 | 0.738 | 0.005 |
| Antibacterial Drugs | 1 | 0 | 0.928 | 0.004 |
| Antibiotics | 116 | 0 | 0.983 | 0.002 |
| Anti-helicobacter pylori agents | 1 | 0 | 0.202 | 0.091 |
| Antimycobacterial agents | 13 | 0 | 0.516 | 0.014 |
| Endometriosis therapy | 7 | 7 | | |
| GnRH (LHRH) antagonists | 18 | 0 | 0.587 | 0.002 |
| Macrolides | 256 | 0 | 0.993 | 0.001 |
| Motilin receptor agonists | 11 | 0 | 0.343 | 0.001 |
| NF-(kappa)B activation inhibitors | 1 | 1 | | |
| Oncolytic drugs | 11 | 0 | 0.427 | 0.095 |
| Prokinetic agents | 11 | 0 | 0.858 | 0.002 |
| Prostate cancer therapy | 7 | 0 | 0.584 | 0.003 |
| Quinolones | 9 | 9 | | |
| Treatment of protozoal diseases | 1 | 0 | 0.565 | 0.012 |
| Treatment of tuberculosis | 1 | 1 | | |
| Sum | 471 | 18 | 96% | |

Table 1. PASS Prediction of Known Activity Types for 285 Erythromycin Analogues from the MDDR Database[a]

# Combinatorial Biosynthesis

- Parameters:
  - Propionate starter
  - 6 PKS modules
  - 2 glycosylation positions
  - 2 hydroxylation positions

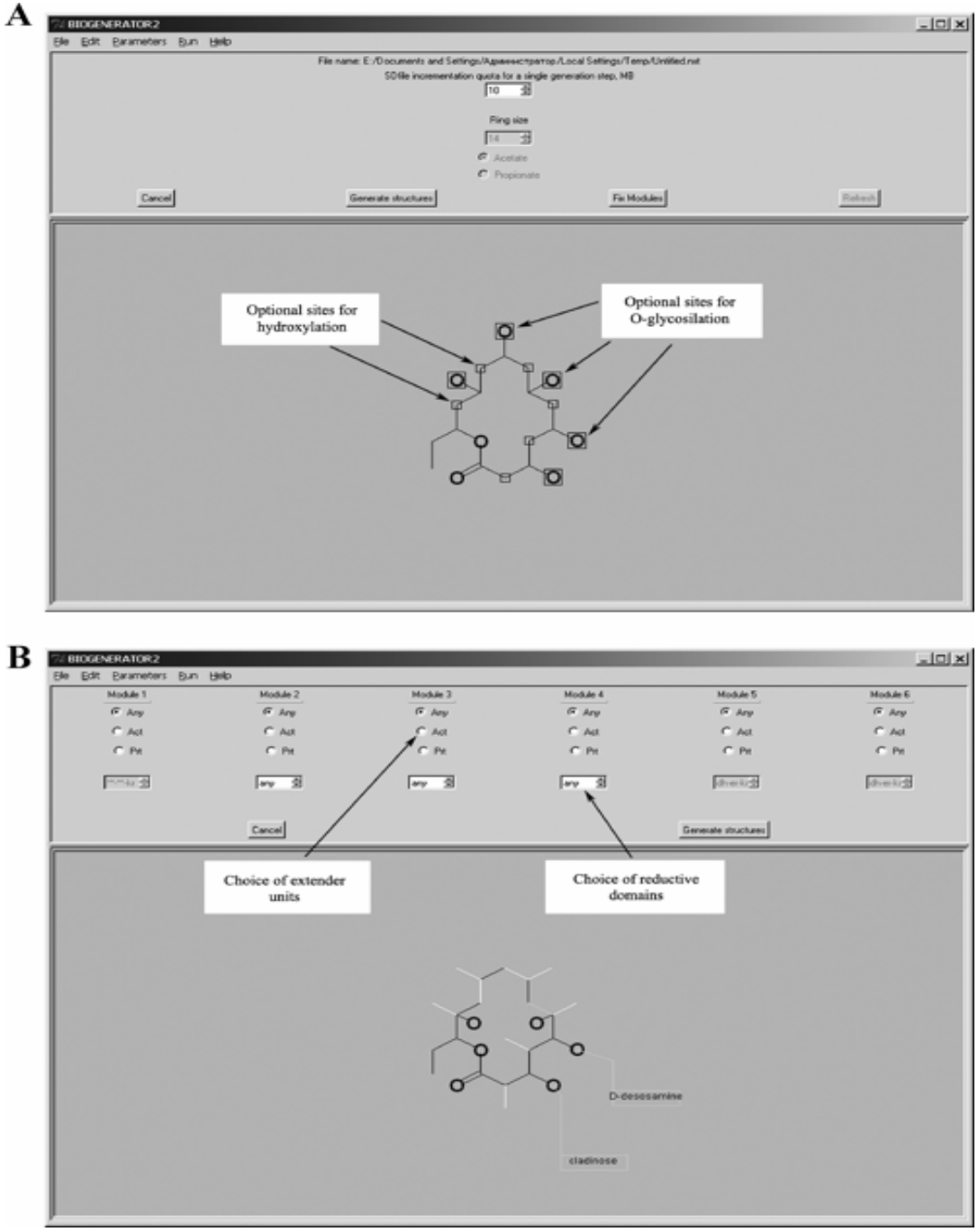
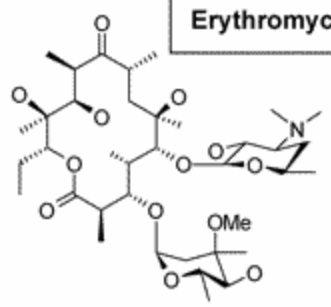



Figure 4, J. Med. Chem, 2006, Vol. 49, No 6

# Results

- 3072 structures
- Pa > 0.7
- Also analyzed 1060 known structures more structurally diverse due to no limitations on additional ring groups

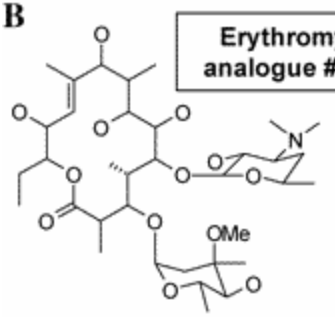
**A**




**PASS ACTIVITY SPECTRUM** at Pa > 0.7

| Pa | Pi | |
|---|---|---|
| 0.975 | 0.003 | Antibacterial |
| **0.970** | **0.004** | **Protein synthesis inhibitor** |
| 0.966 | 0.002 | Protein 50S ribosomal subunit inhibitor |
| 0.952 | 0.004 | HERG channel antagonist |
| 0.864 | 0.007 | CYP3A1 substrate |
| **0.854** | **0.008** | **QT interval prolongation** |
| 0.829 | 0.007 | CYP3A substrate |
| 0.811 | 0.006 | Antidyskinetic |
| 0.783 | 0.006 | Selectin antagonist |
| 0.766 | 0.011 | CYP3A4 substrate |
| 0.743 | 0.010 | Antiprotozoal (Toxoplasma) |
| 0.730 | 0.001 | Testosterone antagonist |

**B**




**[Loading: Propionate]**
**[KS-ATmal-**-**-KR-ACP]**
**[KS-ATmet-DH-**-KR-ACP]**
**[KS-ATmet-**-**-KR-ACP]**
**[KS-ATmal-**-**-KR-ACP]**
**[KS-ATmal-**-**-KR-ACP]**
**[KS-ATmet-**-**-KR-ACP]**

**PASS ACTIVITY SPECTRUM** at Pa > 0.7

| Pa | Pi | |
|---|---|---|
| 0.867 | 0.006 | HERG channel antagonist |
| **0.848** | **0.005** | **Protein synthesis inhibitor** |
| 0.800 | 0.004 | Antimetastatic |
| 0.798 | 0.004 | Antibacterial |
| 0.787 | 0.009 | CYP3A1 substrate |
| 0.758 | 0.021 | Cholesterol synthesis inhibitor |
| 0.746 | 0.010 | Antidyskinetic |
| 0.741 | 0.006 | Selectin antagonist |
| 0.743 | 0.012 | CYP3A substrate |
| **0.562** | **0.056** | **QT interval prolongation** |

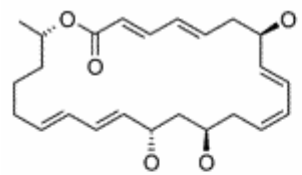
Figure 3, J. Med. Chem, 2006, Vol. 49, No 6

# Example 2: Macrolactin A

- Macrolides with unknown marine origin
- Possible activities: antibacterial, antitumor, shown to inhibit replication of herpes simplex virus and HIV, shown to inhibit cholesterol biosynthesis

# Combinatorial Biosynthesis

- Parameters:
  - Acyl-coA starters
  - Acetate extenders
- Results:
  - 1,048,576 structured library
  - Results similar to known compounds
  - Also introduced new activities



**Macrolactin A PKS:**
**[Loading: Acetate]**

**M1: [KS-ATmal-**-**-KR-ACP]**
**M2: [KS-ATmal-DH-ER-KR-ACP]**
**M3: [KS-ATmal-DH-**-KR-ACP]**
**M4: [KS-ATmal-DH-**-KR-ACP]**
**M5: [KS-ATmal-**-**-KR-ACP]**
**M6: [KS-ATmet-**-**-KR-ACP]**
**M7: [KS-ATmet-DH-**-KR-ACP]**
**M8: [KS-ATmet-DH-**-KR-ACP]**
**M9: [KS-ATmet-**-**-KR-ACP]**
**M10: [KS-ATmet-DH-**-KR-ACP]**
**M11: [KS-ATmet-DH-**-KR-ACP]**

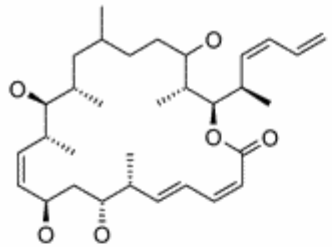
**PASS ACTIVITY SPECTRUM** at Pa > 0.7

| Pa | Pi | |
|---|---|---|
| 0.938 | 0.003 | Cholesterol synthesis inhibitor |
| 0.916 | 0.002 | Antimetastatic |
| 0.784 | 0.008 | H+-transporting two-sector ATPase inhibitor |
| 0.818 | 0.054 | Dextranase inhibitor |
| 0.737 | 0.028 | Antineolpastic (lymphocytic leukemia) |
| 0.737 | 0.028 | Antineolpastic (non-Hodgkin's lymphoma) |
| 0.759 | 0.059 | Phosphatase inhibitor |
| 0.711 | 0.012 | Apoptosis agonist |
| 0.709 | 0.096 | Acylcarnitine hydrolase inhibitor |
| 0.703 | 0.009 | CYP2A11 substrate |

Figure 5, J. Med. Chem, 2006, Vol. 49, No 6

# Results

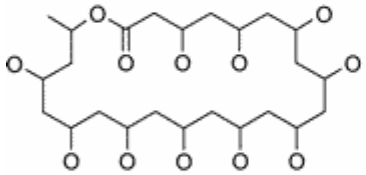
- Macrolactins structurally resemble dictyostatin-1, known to have tubulin stabilizing activity (mechanism of antitumor agents)
- 236 of the macrolactin analogs had microtubule stabilizing activity, Pa> 0.7



**Dictyostatin-1**

**PASS ACTIVITY SPECTRUM** at Pa > 0.7

| Pa | Pi | |
|---|---|---|
| 0.922 | 0.006 | Phosphatase inhibitor |
| 0.895 | 0.005 | Cholesterol synthesis inhibitor |
| 0.827 | 0.006 | H+transporting two -sector ATPase inhibitor |
| 0.803 | 0.004 | Antimetastatic |
| **0.784** | **0.003** | **Microtubule stabilizator** |
| **0.744** | **0.005** | **Microtubule formation stimulant** |
| 0.727 | 0.008 | Immunosuppressant |
| 0.723 | 0.004 | Membrane Permeability enhancer |
| 0.762 | 0.044 | Beta-adrenergic-receptor kinase inhibitor |
| 0.715 | 0.011 | CYP2A substrate |



**Marolactin analogue # 49526**

**PASS ACTIVITY SPECTRUM** at Pa > 0.7

| Pa | Pi | |
|---|---|---|
| 0.951 | 0.003 | Cholesterol synthesis inhibitor |
| 0.929 | 0.004 | Antineolpastic (lymphocytic leukemia) |
| 0.929 | 0.004 | Antineolpastic (non -Hodgkin's lymphoma) |
| 0.910 | 0.016 | Dextranase inhibitor |
| 0.863 | 0.003 | Antimetastatic |
| 0.845 | 0.040 | Acylcarnitine hydrolase Inhibitor |
| 0.778 | 0.052 | Flavanone 4-reductase inhibitor |
| 0.773 | 0.015 | Membrane integrity Antagonist |
| 0.771 | 0.054 | Phosphatase inhibitor |
| 0.762 | 0.007 | Apoptosis agonist |
| 0.759 | 0.006 | Pectin lyase inhibitor |
| **0.749** | **0.003** | **Microtubule stabilizator** |
| 0.755 | 0.012 | Bcl2 antagonist |
| 0.745 | 0.007 | CYP3A5 substrate |
| 0.747 | 0.016 | Glycerol-1-phosphatase inhibitor |
| 0.736 | 0.010 | Atherosclerosis treatment |

Figure 6, J. Med. Chem, 2006, Vol. 49, No 6

# Conclusions

- Results of the software give “instructions” for molecular biologists to follow in the construction of desired products
- Reasonable accuracy
- Currently experimentally testing some samples